

# Dot blot assay

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Inhibition of METTL3 attenuates renal injury and inflammation by alleviating TAB3 m6A modifications via IGF2BP2-dependent mechanisms

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## Detailed protocol

### Dot blot assay

An RNA m6A dot blot assay was used to measure the m6A content in the poly-A tailing of total RNA. In brief, total RNA was isolated using TRIzol (Invitrogen, 15596018) following the manufacturer's instructions. The RNAs (100 and 200 ng respectively) were double diluted and spotted onto a nylon membrane (Sigma-Aldrich, GERPN1210B). Then the membranes were ultraviolet crosslinked and blocked in 5% nonfat milk, then was incubated with the m6A antibody (Abcam) overnight. The membranes were finally incubated with the secondary antibody at room temperature for 1 hour. Signals were detected using LI-COR/Odyssey infrared image system (LI-COR Biosciences). Then 0.02% methylene blue in 0.3 M sodium acetate (pH 5.2) was used to show the amount of total RNA.

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

- Wu, Y. and Meng, X. (2022). Dot blot assay. Bio-protocol Preprint. [bio-protocol.org/prep1648](https://bio-protocol.org/prep1648).
- Wang, J., Wang, F., Ke, J., Li, Z., Xu, C., Yang, Q., Chen, X., He, X., He, Y., Suo, X., Li, C., Yu, J., Jiang, L., Ni, W., Jin, J., Liu, M., Shao, W., Yang, C., Gong, Q., Chen, H., Li, J., Wu, Y. and Meng, X. (2022). Inhibition of METTL3 attenuates renal injury and inflammation by alleviating TAB3 m6A modifications via IGF2BP2-dependent mechanisms. Science Translational Medicine 14(640). DOI: [10.1126/scitranslmed.abk2709](https://doi.org/10.1126/scitranslmed.abk2709)

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